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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/622,240	07/18/2003	George Tzertzinis	NEB-208/9-US	3580

28986 7590 11/25/2009

HARRIET M. STRIMPEL, D. Phil.
New England Biolabs, Inc.
240 COUNTY ROAD
IPSWICH, MA 01938-2723

EXAMINER	
POPA, ILEANA	

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
11/25/2009	ELECTRONIC

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STRIMPEL@NEB.COM
Goldberg@neb.com
wermuth@neb.com

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte GEORGE TZERTZINIS,
GEORGE FEEHERY, CORINNA TUCKEY,
CHRISTOPHER NOREN, and LARRY MCREYNOLDS

Appeal 2009-004205[1]
Application 10/622,240
Technology Center 1600

Decided:[2] November 23, 2009

Before DEMETRA J. MILLS, LORA M. GREEN, and
FRANCISCO C. PRATS, *Administrative Patent Judges*.

PRATS, *Administrative Patent Judge*.

DECISION ON APPEAL

This appeal under 35 U.S.C. § 134 involves claims to methods of producing a plurality of double stranded RNA fragments. The Examiner has

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[1] New England Biolabs, Inc. is the real party in interest.
[2] Oral argument was presented in this case on November 5, 2009.

rejected the claims as obvious. We have jurisdiction under 35 U.S.C. § 6(b).

We reverse.

STATEMENT OF THE CASE

"RNA interference (RNAi) employing short double-stranded RNA (siRNA) is a powerful tool for silencing gene expression in mammalian cells" (Spec. 1). One prior art "approach for generating small double-stranded RNAs has been to use *E.coli* RNaseIII in the presence of magnesium ions to partially digest large double-stranded RNA" (*id.* at 3-4).

However, "[u]nless digestion is carefully monitored, RNaseIII in the presence of magnesium ions cleaves large double-stranded RNA into very small fragments that are generally considered to have no known use in RNAi" (*id.* at 4). Thus, "[p]roblems associated with this approach include low recovery amounts of the double-stranded fragments in a specific size range larger than about 15nt and the associated inconvenience of titration to avoid over or under- digestion" (*id.*).

The Specification discloses a method of "producing a heterogeneous siRNA (hsiRNA) mixture, that includes digesting a preparation of large double-stranded RNA in a reaction mixture containing a divalent transition metal cation and RNaseIII . . . Examples of transition metal cations for use in making hsiRNA mixtures include manganese, nickel, cobalt, zinc and cadmium" (*id.* at 5). According to the Specification, the disclosed methods provide "an enhanced concentration of double-stranded RNA fragments of a size suitable for silencing of gene expression by a rapid, cost effective process that is not dependent on a gel based size separation step" (*id.* at 22).

Claims 1, 2, 5-7, 9, 12-14, 16-18, 20, and 47 stand rejected and are on appeal (App. Br. 2). Claims 1 and 13, the independent claims, are representative and read as follows:

> 1. A method of producing a plurality of overlapping double stranded (ds) RNA fragments of a size in the range of about 15-30 nucleotides, comprising:
>
> (a) digesting a preparation of large double-stranded RNA in a reaction mixture containing a divalent transition metal cation and a prokaryotic RNaseIII wherein the ratio of enzyme to substrate (w/w) is greater than or equal to about 0.25:1; and
>
> (b) producing the plurality of overlapping dsRNA fragments of a size in the range of about 15-30 nucleotides.
>
> 13. A purified set of double-stranded RNA fragments, comprising a plurality of overlapping fragments of a size in the range of about 15-30 nucleotides, the fragments in the set collectively representing a substantial portion of a sequence of one or more large double-stranded RNAs from which the fragments are derived by in vitro cleavage with a purified enzyme, one strand of each of the large double-stranded RNA having a sequence complementary to part or all of a target RNA.

The Examiner cites the following documents as evidence of unpatentability:

Yang	US 2004/0014113 A1	Jan. 22, 2004

G. Gross et al., *Structure of secondary cleavage sites of E. coli RNAaseII in A3t RNA from bacteriophage T7*, 15 Nucleic Acids Research 431-442 (1987).

The sole rejection before us for review is the rejection of claims 1, 2, 5-7, 9, 12-14, 16-18, 20, and 47, as being unpatentable under 35 U.S.C. § 103(a) in view of Yang and Gross (Ans. 3-5).

OBVIOUSNESS

ISSUE

The Examiner cites Yang as describing a method having the claimed process steps, one difference being that Yang's reaction mixture contains magnesium rather than a divalent transition metal cation such as manganese (Ans. 4). To meet that limitation the Examiner cites Gross as teaching that "replacing magnesium with manganese promotes the cleavage of additional sites in the double-stranded RNAs" (*id.* at 5).

Based on these teachings, the Examiner reasons, an ordinary artisan would have been prompted to replace Yang's magnesium with Gross' manganese, as the artisan would have understood from Gross' teachings that "the use of manganese results in a higher cleavage efficiency" for RNase III (*id.*).

The Examiner also concedes that Yang's process differs from the claimed process with respect to the claimed enzyme:substrate ratio, but reasons that it would have been obvious for an ordinary artisan "to vary the parameters in a given method with the purpose of optimizing the results; it is generally not inventive to discover the optimal working conditions of a prior art method, such conditions can be identified by routine experimentation" (*id.*).

Appellants contend that "the Examiner has failed to establish even a *prima facie* case of unpatentability" (App. Br. 5). Among other things, with respect to claim 1, Appellants argue that

> [n]o sound reason has been articulated for changing the limited digestion of Yang by <u>increasing</u> the amount of enzyme by at least 20-fold and using a divalent transition metal cation, nor was there a sound basis for concluding that this would lead

> to a desirable method for producing overlapping dsRNA fragments of a size in the range of about 15-30 nucleotides.

(*Id.* at 7.)

Moreover, Appellants argue, given Yang's disclosure of the undesirability of excessively cleaving the larger dsRNA starting material, an ordinary artisan viewing Gross' disclosure that manganese promotes RNA cleavage at additional sites would not have been prompted to use manganese in Yang's reaction, "as that would only *exacerbate* the problems Yang was trying to address by limiting the digestion" (*id.* at 8).

With respect to claim 13, Appellants similarly argue that "[t]he initial burden is on the Examiner to establish a *prima facie* case of unpatentability. Here, the Examiner has failed to explain why one of ordinary skill in the art would find the claimed invention predictable in view of the references" (*id.* at 14).

In view of the positions advanced by Appellants and the Examiner, the issue with respect to this rejection is whether Appellants have shown that the Examiner failed to make a prima facie case that an ordinary artisan would have considered the claimed processes and products obvious in view of Yang and Gross.

FINDINGS OF FACT ("FF")

1. Yang discloses a process in which "E. coli RNase III is used to cleave double-stranded RNA into esiRNA (endoribonuclease-prepared siRNA) that can target multiple sites within an mRNA. The invention therefore provides an RNA duplex pool that can recognize multiple sites in any particular RNA to silence gene expression" (Yang [0004]).

2. Yang discloses, however:

> Exhaustive cleavage of dsRNA by E. coli RNase III leads to duplex products averaging 12-15 bp in length. These short dsRNA are unable to trigger an RNAi response in mammalian cells. To obtain siRNA of appropriate length . . . limited RNase III digestion of dsRNA was performed, efficiently generating 20-25 bp siRNA.

(*Id.* at [0015] (citations omitted).)

3. Regarding its process, Yang further discloses:

> After optimization, we found that limited RNase III digestion of dsRNA at room temperature for one hour yielded ample amounts of esiRNA for inhibition of most genes. Efficiently processing dsRNA with high sequence complexity into short species is consistent with the lack of sequence-specificity in substrate recognition and cleavage by E. coli RNase III. We separated RNase III-digestion products on polyacrylamid[e] gels and purified the RNAs corresponding to approximately 21-23, 24-26, and 27-30 bp (FIG. 1c). For simplicity, we named siRNA prepared by RNase III digestion as esiRNA (endoribonuclease-prepared siRNA).

(*Id.* at [0053] (citation omitted).)

4. Yang describes the specifics of its reactions as follows:

> To prepare esiRNAs for F-luc and R-luc [(recombinant luciferase genes to be silenced)], 100 μg of dsRNAs were digested by 1 μg of recombinant RNase III in a 200 μl reaction buffer (same as dialysis buffer except 5% glycerol) for 15 min at 37° C. Reactions were terminated by adding EDTA to 20 mM and the products separated on 12% polyacrylamide gel, 1xTBE. A 10 bp DNA marker was used to estimate the migration of RNA duplexes. Short RNAs of appropriate sizes were eluted from gel slices by soaking in 1 M $(NH_4)_2AC$ at 37°C over night and recovered by ethanol precipitation. The precipitate was dissolved in TE buffer at 1.0 μg/μl. For other genes, 100 μg of dsRNAs were digested with 0.2 μg of RNase

> III for one hour at 21°C and reactions were loaded onto QIAquick spin columns after being supplemented with 5 volume of PN buffer (QIAquick nucleotide removal kit, Qiagen). Flowthrough usually contained RNA from 15 to 25 base paires [sic], which was precipitated by ethanol and dissolved in TE buffer.

(*Id.* at [0079].)

5. Yang's dialysis buffer, used for hydrolyzing dsRNA, contained "20 mM TrisHCL, 0.5 mM EDTA, 5 mM MgCL2, 1 mM DTT, 140 mM NaCL, 2.7 mM KCL, 30% glycerol, [at] pH 7.9" (*id.* at [0074]).

6. Gross discloses:

> E.coli RNAaseIII introduces homogenous cuts in A3t RNA (141 bases) from bacteriophage T7 at relaxed (< 0,1 M) monovalent salt concentrations apparently at a relatively short stretch of base pairing in ssRNA. Additional secondary sites are cut in the presence of manganese (Mn^{2+}) instead of magnesium (Mg^{2+}) ions. RNAaseIII may therefore be used as a trimming tool for RNA molecules giving homogenous 5-ends.

(Gross 432.)

7. Gross discloses:

> RNAaseIII cleaves some ssRNAs differently in the presence of manganese (Mn^{2+}) instead of magnesium (Mg^{2+}) (Fig. 4a). Here we show that in a RNA molecule containing one primary RNAaseIII site (here: between A3t and 0.3 RNA) a different cleavage pattern occurs depending on the cation used (Mg^{2+}/ Mn^{2+}). Primary sites are efficiently cleaved in the presence of Mg^{2+} between 0.1 M - 0.3 M NaCl and in the presence of Mn^{2+} from 0.3 M - 0.5 M NaCl. Secondary sites, however, are cleaved differently in the presence of Mn^{2+} instead of Mg^{2+} depending on the monovalent salt concentration. In 5'-endlabeled A3t RNA we localized two Mn^{2+} dependent secondary sites (Fig. 4b). . . . Therefore E.coli RNAaseIII cleavage in ssRNA can be influenced as well as by changing of

> monovalent salt concentration as by changing of divalent metal ions.

(Gross 439.)

PRINCIPLES OF LAW

> In proceedings before the Patent and Trademark Office, the Examiner bears the burden of establishing a *prima facie* case of obviousness based upon the prior art. "[The Examiner] can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references."

In re Fritch, 972 F.2d 1260, 1265 (Fed. Cir. 1992) (citations omitted, bracketed material in original).

In *KSR Int' l Co. v. Teleflex Inc.*, 550 U.S. 398, 415 (2007), the Supreme Court emphasized "an expansive and flexible approach" to the obviousness question. The Court nonetheless reaffirmed that "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *Id.* at 418.

Rather, as the Court stated:

> [I]t can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements *in the way the claimed new invention does* . . . because inventions in most, if not all, instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known.

Id. at 418-419 (emphasis added); *see also id.* at 418 (requiring a determination of "whether there was an apparent reason to combine the

known elements *in the fashion claimed* by the patent at issue") (emphasis added).

Ultimately, therefore, as our reviewing court has stated, "[i]n determining whether obviousness is established by combining the teachings of the prior art, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art." *In re GPAC Inc.*, 57 F.3d 1573, 1581 (Fed. Cir. 1995) (internal quotations omitted).

When evaluating claims for obviousness, "the prior art as a whole must be considered. The teachings are to be viewed as they would have been viewed by one of ordinary skill." *In re Hedges*, 783 F.2d 1038, 1041 (Fed. Cir. 1986).

Moreover, "obviousness requires a suggestion of all limitations in a claim." *CFMT, Inc. v. YieldUp Int'l. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (citing *In re Royka*, 490 F.2d 981, 985 (CCPA 1974)).

ANALYSIS

We agree with Appellants that the Examiner failed to make a prima facie case that an ordinary artisan would have considered the claimed processes and products obvious in view of Yang and Gross.

With respect to the process recited in claim 1, the greatest amount of enzyme used by Yang in its process is one microgram of RNase III per one hundred micrograms of substrate, for fifteen minutes of reaction time (FF 4). As the Examiner concedes (Ans. 12), this equates to an enzyme:substrate ratio of 0.01:1. Thus, the claimed ratio of at least 0.25:1 is twenty five times greater than the amount used by Yang.

As the Examiner argues (Ans. 12-13), it might be true that optimizing Yang's process when manganese is used as a cofactor instead of magnesium

would yield a different suitable range of enzyme:substrate values. However, in view of Gross' disclosure that RNase III cleaves single stranded RNA at more sites in the presence of manganese, as compared to magnesium (FF 6, 7), it appears that an ordinary artisan would have expected Yang's process to require less enzyme, rather than more, when using manganese as a cofactor.

Moreover, Yang describes its process conditions as having already been "optimiz[ed]" to yield the desired fragment sizes (FF 3), and to avoid over-digesting the starting material and the undesirable inactive smaller fragments (FF 2). We are therefore not persuaded that an ordinary artisan further optimizing Yang's process would have arrived at an enzyme:substrate ratio twenty five times that disclosed in Yang, even when optimizing with manganese in the reaction mixture.

Nor has the Examiner pointed to any explicit suggestion in the prior art suggesting the desirability, or even suitability, of increasing the amount of enzyme relative to substrate in Yang's process, to the degree required in claim 1. Rather, given Yang's disclosure that its process was already optimized to avoid excessive digestion of the starting material, we agree with Appellants that an ordinary artisan would not have been prompted to increase the enzyme:substrate ratio to the degree claimed.

Thus, because we agree with Appellants that the combination of Yang and Gross fails to teach or suggest all of the features in claim 1, we reverse the Examiner's rejection of that claim, and its dependents, as being obvious over those references.

With respect to claim 13, Appellants argue that the Examiner "has presented no evidence that one of ordinary skill in the art would have expected to produce overlapping fragments collectively representing a

substantial portion of a sequence of one or more large double-stranded RNAs" (App. Br. 14). The Examiner states only that the "response to this argument is the same as set forth above for claim 1" (Ans. 17).

Thus, while the Examiner's rationale with respect to claim 13 is less than readily apparent, the Examiner does state that

> it is not clear why the same *E. coli* RNase III would be able to produce overlapping fragments when used in the instant method and not be able to do so in the method taught by Yang et al. and Gross et al., which is similar to the instant method; the method of Yang et al. and Gross et al. would necessarily result in overlapping fragments representing a substantial portion of the RNA from which they are derived, because the RNase III sites are spread over the entire lengths of the RNAs. Therefore, Applicant only observed an inherent property of RNase III and this cannot be considered an unexpected result.

(*Id.* at 14.)

We agree with Appellants that the Examiner failed to make a prima facie case of obviousness with respect to claim 13. We note, as the Examiner points out, that RNase III sites are spread over the entire length of the larger dsRNA starting material. However, once the starting material is digested, Yang purifies dsRNA within the claimed size range, from either gel slices or "QIAquick spin columns" (FF 4).

Thus, while it may be true that the total digest of the starting material collectively represents the starting material's sequence, claim 13 recites a set of purified set of RNA fragments, and the Examiner has not adequately explained why Yang's purified fragments constitute a substantial portion of either of the two strands from which the smaller fragments were derived, as required by claim 13. Moreover, given the fact that RNase III behaves differently in the presence of manganese, as compared to magnesium (FF 6,

7), we agree with Appellants that the Examiner has not provided adequate evidence to conclude that using manganese in Yang's process would inherently produce the set of fragments recited in claim 13.

Accordingly, we also agree with Appellants that the Examiner has failed to make a prima facie case that the combined disclosures of Yang and Gross teach or suggest the product recited in claim 13. We therefore reverse the Examiner's rejection of claim 13 and its dependent claims.

SUMMARY

We reverse the Examiner's rejection of claims 1, 2, 5-7, 9, 12-14, 16-18, 20, and 47 as being unpatentable under 35 U.S.C. § 103(a) in view of Yang and Gross.

REVERSED

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HARRIET M. STRIMPEL. D. PHIL.
NEW ENGLAND BIOLABS, INC.
240 COUNTY ROAD
IPSWICH, MA 01938-2723